

Appl. No. : 10/621009
Filed : July 15, 2003

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

At the paragraph at page 1, lines 5-11:

This application is a continuation of and claims priority to U.S. patent application Ser. No. 09/932,129, filed Aug. 16, 2001, now abandoned, which is a continuation of and claims priority to International Application No. PCT/US00/09230, filed Apr. 7, 2000, designating the United States of America and published in English, which claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/128,378, filed Apr. 8, 1999; all of which are hereby expressly incorporated by reference in their entireties.

At the paragraphs that were added to the specification below page 3, line 3 in the amendment dated June 28, 2005:

The present invention also provides a method for amplifying a target nucleic acid sequence, comprising the steps of: combining said target nucleic acid sequence a forward anchor (FA), forward primer (FP), reverse anchor (RA), reverse primer (RP), forward universal primer (FUP) and reverse universal primer (RUP), wherein said FA/FP readily associate to form a first primer pair and said RA/RP readily associate to form a second primer pair via association of their complementary stem regions in the absence of said target nucleic acid, wherein said FUP is complementary to the FA/FP stem region, and wherein said RUP is complementary to the RA/RP stem region wherein said primer pairs are selected on the basis of complementarity to said target nucleic acid sequence to flank said target nucleic acid sequence; and amplifying said nucleic acid sequence via enzyme-mediated amplification. In one embodiment, said nucleic acid sequence encodes a therapeutic gene product. In another embodiment, said nucleic acid sequence is DNA or RNA. In another embodiment, said enzyme mediated amplification is PCR amplification.

The present invention also provides a method for amplifying a target nucleic acid sequence, comprising: providing a first oligonucleotide primer comprising a first target binding region configured to bind to a first end of said target nucleic acid sequence, and a first stem region, wherein said first stem region comprises a first universal primer binding region;

providing a second oligonucleotide primer comprising a second target binding region configured to bind to said first end of said target nucleic acid sequence, and a second stem region that is homologous with said first stem region, so that said first oligonucleotide primer and said second oligonucleotide primer join together to form a first primer pair that binds to said target nucleic acid sequence; providing a universal primer configured to bind with said first universal primer binding region; providing an additional primer configured to bind with a second end of said target nucleic acid sequence; and incubating said first primer pair, universal primer, and additional primer in the presence of said target nucleic acid sequence under conditions that amplify said target nucleic acid. In one embodiment, the first stem region and the first target binding region are connected by a flexible linker. In another embodiment, the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters. In another embodiment, the second stem region and the second target binding region are connected by a flexible linker. In another embodiment, the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters. In another embodiment, the first oligonucleotide primer comprises one or more modified bases. In another embodiment, the second oligonucleotide primer comprises one or more modified bases. In another embodiment, the target nucleic acid sequence is DNA. In another embodiment, the target nucleic acid sequence is RNA. In another embodiment, said incubating comprises the Polymerase Chain Reaction. In another embodiment, said amplifying is mediated by an enzyme selected from the group consisting of: Taq polymerase and reverse transcriptase. In another embodiment, said additional primer comprises: a third oligonucleotide primer comprising a third target binding region configured to bind to a second end of said target nucleic acid sequence, a reverse transcriptase binding region, and a third stem region, wherein said third stem region comprises a second universal primer binding region; and a fourth oligonucleotide primer comprising a fourth target binding region configured to bind to said second end of said target nucleic acid sequence, and a fourth stem region that is homologous with said third stem region, so that said third oligonucleotide primer and said fourth oligonucleotide primer join together to form a second primer pair that binds to said target nucleic acid sequence.

The present invention also provides a method of amplifying a target nucleic acid sequence, comprising the steps of: providing a target nucleic acid sequence having a first amplification region and a second amplification region; selecting from a library of first oligonucleotide primers, a first oligonucleotide primer that comprises a first target binding region that is homologous with a portion of the first amplification region, wherein said first oligonucleotide primer comprises a first stem region and a first universal primer binding region; selecting from a library of second oligonucleotide primers, a second oligonucleotide primer that comprises a second target binding region that is homologous with a second portion of the first amplification region, wherein said second oligonucleotide primer comprises a second stem region that is homologous with said first stem region, so that said first oligonucleotide primer and said second oligonucleotide primer join together to form a first primer pair that binds to said target nucleic acid sequence at said first amplification region; providing a universal primer configured to bind with said first universal primer binding region; providing an additional primer configured to bind with a second amplification region of said target nucleic acid sequence; and incubating said first primer pair, universal primer, and additional primer in the presence of said target nucleic acid sequence under conditions that amplify said target nucleic acid. In one embodiment, the library of first oligonucleotide primers comprises greater than 65,536 oligonucleotides. In another embodiment, the first target binding region comprises at least six nucleotides. In another embodiment, the second target binding region comprises at least six nucleotides. In another embodiment, the first target binding region and the first stem region are connected by a flexible linker. In another embodiment, the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters. In another embodiment, the second target binding region and the second stem region are connected by a flexible linker. In another embodiment, the flexible linker is selected from the group consisting of polyethylene glycol, polypropylene glycol, polyethylene, polypropylene, polyamides and polyesters. In another embodiment, the first oligonucleotide primer comprises one or more modified bases. In another embodiment, the second oligonucleotide primer comprises one or more modified bases. In another embodiment, the first oligonucleotide primer comprises one or more modified backbone linkages. In another embodiment, the second oligonucleotide primer comprises one or more modified backbone

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~~linkages. In another embodiment, the target nucleic acid sequence is DNA. In another embodiment, the target nucleic acid sequence is RNA. In another embodiment, said incubating comprises the Polymerase Chain Reaction. In another embodiment, said amplifying is mediated by an enzyme selected from the group consisting of Taq polymerase and reverse transcriptase.~~